The Measurement of Lipopolysaccharide as an Indicator of Bacterial Biomass in the Oligotrophic Pacific Ocean

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Introduction

Measurements of biomass are fundamental in the study of microbial processes in the sea and their relationship to the marine ecosystem. Bacteria are an important component of planktonic food webs (Pomeroy, 1974; Azam et al., 1983) and unicellular food webs are responsible for much of the recycling of photosynthetically produced organic matter (Goldman and Caron, 1985; Ducklow et al., 1986). Bacteria form a substantial portion of the total biomass in the oligotrophic ocean and, with high activity per unit biomass, they may be responsible for 50% or more of total ecosystem respiration.

A large majority of carbon in the ocean exists in dissolved form and only a fraction of particulate carbon is associated with living organisms, including bacteria. Therefore, accurate measurements of bacterial biomass can be very difficult to make. Methods for the estimation of microbial biomass in the sea involve target molecules such as: ATP (adenosine triphosphate) for all living microorganisms, Chl *a* for photoautotrophic microbes, phospholipids for all living microorganisms, ergosterol for fungi, lipopolysaccharide (LPS) for gram-negative bacteria, and muramic acid for all bacteria (Karl and Dobbs, submitted 1996; Karl, 1986). Direct counts of bacterial cells can also be used to estimate biomass, but variation in cell size makes the estimation very crude. Benefits and limitations associated with each method are outlined by Karl and Dobbs (submitted to Molecular Approaches to the Study of the Ocean K. E. Cooksey, Ed., 1996). This project focused on the use of the LPS method to detect the biomass of Gram-negative bacteria in the oligotrophic ocean north of Hawaii.

Lipopolysaccharides, otherwise known as endotoxins or pyrogens, are constituents of the outer cell membranes of Gram-negative bacteria which

make up 80-95% of marine prokaryotes. LPS is detected in water samples through the application of the *Limulus* Amebocyte Lysate (LAL) assay which was developed by Levin and Bang (1964). Amebocytes extracted from the horseshoe crab *Limulus polyphemus* form clots in the presence of endotoxins, causing the solution to become turbid at a rate that is directly proportional to the amount of endotoxin present. The quantity of LPS in a solution is determined by the use of a spectrophotometer to measure the rate and the extent of the change in turbidity.

In order for an accurate measurement of bacterial biomass to be made, the amount of total LPS that is associated with cells must be separated from dissolved LPS is present in virtually all seawater. The standard separation procedure involves centrifuging the sample water for 10 minutes and storing the supernatant, or dissolved LPS, in a separate container. Particulate LPS, or that associated with intact cells, is then defined as the total LPS minus the dissolved LPS. Although this is the accepted method for separating dissolved from particulate LPS there is a lack of documentation supporting its effectiveness. I compared the quantities of LPS in samples run through different types of filters and centrifuged for different periods of time in order to determine the most effective way to separate dissolved from particulate LPS.

The assay for detecting LPS in solution is very sensitive, making it necessary to dilute most seawater samples before analyzing them. The correct level of dilution is important to insure an accurate measure of the LPS content. If a sample is diluted too much a false negative result could be obtained, and if the sample is not diluted enough the turbidity of the sample water would increase too quickly for an accurate measure of LPS to be made. LPS content was measured in samples prepared at different levels of dilution,

with a sample spiked with a known quantity of LPS included for each different level of dilution and each depth range analyzed. By subtracting the amount of LPS detected in the unspiked sample from that of the spiked sample and assessing the efficiency of the spike return, the accuracy of the measurements at each dilution were monitored.

Since the assay is so sensitive, the integrity of the storage containers is important. Roslansky et al. (1991) reported that polypropylene containers made by certain manufacturers can cause an inhibition of the LPS signal and recommended that containers be tested before they are used for LPS samples. I tested five different types of containers for effects on the detected LPS signal.

It is thought that bacterial cells contain LPS in relatively constant proportions to carbon, making it an excellent indicator of biomass (Rheinheimer, 1974; ZoBell and Upham, 1944). Currently a C:LPS ratio of 6.35 is used to convert quantities of measured particulate LPS into units of biomass. This conversion factor, derived by Watson *et al.* (1977), is based on a study that involved cultures of *Escherichia coli* and was validated with a field study in coastal waters off the coast of Africa. Giovannoni *et al.* (1990) point out that natural populations of marine bacterioplankton are very diverse and very little is known about the species within them. Although a conversion factor based on a culture of *E. coli* may be applicable to a rich coastal environment, it is questionable whether it would be relevant in an oligotrophic environment where, at the very least, the bacterial cells are likely much smaller. I plotted profiles of LPS abundance in relation to depth from samples taken on two cruises in the oligotrophic Pacific Ocean north of the island of Oahu, Hawaii. I compared biomass estimations based on the LPS method and Watson's conversion factor to estimations based on the numbers of bacteria (flow cytometry) and on the ATP method in order to assess the

applicability of the conversion factor. I also plotted the numbers of bacteria against the amount of LPS detected to illustrate the relationship between them.

Materials and Methods

LPS test.

I measured the quantity of LPS in water samples using the turbidimetric method of the LAL assay with an LAL-5000 endotoxin detection and data analysis system. Freeze-dried LAL and control standard endotoxin (CSE) were obtained from the Associates of Cape Cod, Inc. (Woods Hole, Mass.). I reconstituted the CSE with pyrogen-free deionized water and stored it in the refrigerator between uses for no longer than four weeks and used pyrogen-free seawater to reconstitute LAL on each day of analysis. Any left-over LAL was stored in the freezer, but was never frozen more than once. UV-oxidized seawater, free of endotoxin, was used for sample blanks, standard curve dilutions, and sample dilutions. A standard curve of 0.1, 0.05, 0.01, 0.005, 0.001, and 0.0005 ng of control standard endotoxin per milliliter of pyrogen-free seawater was prepared for each run. All glassware used in this assay was rendered LPS-free by heating at 250°C for at least three hours.

Each run of the assay included two sample blanks, a standard curve, and samples prepared at the appropriate dilutions in 10 x 75 mm borosilicate glass tubes (Fisher Scientific Co.). For each different level of dilution and each different type of sample, a spiked sample containing an addition of 0.025 ng control standard endotoxin was included to insure accurate detection of LPS. I added 100 µl of LAL to each glass tube, containing a total volume of 400 µl, mixed it briefly with a vortex mixer and inserted it into the

spectrophotometer portion of the LAL-5000 endotoxin detection and data analysis system.

Comparison of separation methods.

I compared filtration and centrifugation methods for their relative effectiveness at separating dissolved from particulate LPS. Surface seawater was filtered through GF/F, 0.2 μm Nuclepore, and 0.4 μm Nuclepore filters and collected in an acid washed Millipore filtration unit. Seawater from the same source was centrifuged at 13,000 x g for 5, 10, 15, and 20 minutes in a 1.5 ml Fisherbrand microcentrifuge tube and the supernatant was transferred into a new tube. The quantities of LPS in each of these samples and in the unprocessed seawater were compared and the most effective method for separating dissolved from particulate LPS was defined as the one which removed the most cells, or the one that resulted with the lowest level of dissolved LPS.

Comparison of dilution levels.

All dilutions were made with pyrogen-free seawater. Duplicate samples, plus a spiked sample (0.025 ng of LPS added) for each set, from different depths were tested for LPS quantity at dilution levels of 1:5, 1:10, and 1:20. The appropriate level of dilution was determined for each depth range by comparing the spike return efficiency at the different levels of dilution.

Comparison of containers.

Coastal seawater from a site near Ala Moana beach on the south shore of Oahu and was tested for "time-zero" total and particulate LPS levels within two hours after collection. Ten samples of dissolved LPS were stored frozen in 1.5 ml microcentrifuge tubes (Fisher Scientific) and ten samples of total LPS were stored frozen in five different types of containers: polypropylene (Corning, 15 ml), polypropylene (Becton Dickson), polystyrene (Becton

Dickson), polypropylene (Fisher Scientific ,1.5 ml microcentrifuge tube), and borosilicate glass (Fisher Scientific). Samples were tested for LPS content after 1 day, 2 days, 3 days, 14 days, and 15 days.

Seawater samples.

Samples were collected at the Hawaii Ocean Time (HOT) series station ALOHA (A Long-term Oligotrophic Habitat Assessment), 100 km north of the island of Oahu on the HOT 73 cruise (June 24th through the 28th) and a 5 sites along a transect line that included station ALOHA on the ALOHA-CLIMAX Transect cruise (July 8th through the 16th). Water was collected with a conductivity-temperature-depth (CTD)/rosette sampler outfitted with twenty-four 12-L Niskin-type bottles from which samples were drawn into 15-ml Corning centrifuge tubes (pyrogen-free). Total and particulate LPS were separated by centrifuging 1 ml samples for 10 min. at 13,000 x g. On most, but not all, casts total LPS samples were stored in both the 15 ml polypropylene tubes used for collection and in 1.5 ml polypropylene microcentrifuge tubes. Dissolved LPS samples were stored in the 1.5 ml microcentrifuge tubes. All samples were stored frozen until they were analyzed.

Samples for LPS were taken from the same casts and the same depths at which ATP, Chl *a*, and particulate carbon and nitrogen were taken. Subsamples of 1 ml were transferred, in duplicate, to Corning cryogenic vials preloaded with 20 µl of paraformaldehyde and then stored in liquid Nitrogen for the enumeration of heterotrophic bacteria by the dual-laser flow cytometric method of Monger and Landry (1993).

Biomass estimations based on measurements of ATP, flow cytometry, and particulate LPS were calculated for the ATP cast of the HOT 73 cruise and for station 2 of the Transect cruise. Conversion to units of carbon were made using current factors of 250 for ATP, 6.35 for LPS, and both 10 ng and 6.25 ng of

carbon per cell for bacterial cell numbers. Quantities of particulate LPS were plotted against the corresponding number of heterotrophic bacteria on a log-log scale.

Results

Methods of separation.

The amounts of LPS measured in the different filtrates and centrifuged samples are shown in Figure 1. Filtration through the GF/F filter resulted in a quantity of LPS that was higher than the total quantity of LPS measured. Nuclepore filters with pore sizes of 0.2 and 0.4 μm produced similar quantities of dissolved LPS, both of which were lower than that produced with the GF/F filter. All of the centrifuged samples contained similar amounts of LPS, which were lower than the amounts detected in the filtered samples.

Levels of dilution.

Based on the efficiency of the spike returns the appropriate levels of dilution were determined to be: 1:20 for total and 1:10 for dissolved for water collected between the surface and 200 m in depth; 1:10 for total and 1:5 for dissolved for water between 200-500 m; and 1:5 for total and 1:2 for dissolved for water deeper than 500 m.

Effects of containers.

The quantities of LPS detected in each container at each time point is shown in Figure 2. There was a large increase in the amount of LPS detected in the 15 ml polypropylene tube (PP (large)), the Becton Dickson polypropylene tube (PP (med.)), and the polystyrene tube on Day 2. The quantities of LPS detected on subsequent days returned to levels closer to the original quantity so the increase on Day 2 was probably the result of contamination. Taking into account the reported 25% variation inherent in

the analysis (Associates of Cape Cod), the containers tested did not appear to effect the level of LPS detected.

Depth profiles.

The amounts of particulate LPS found at station ALOHA on HOT 73 and at the five stations along the ALOHA-CLIMAX transect are shown in Figures 3-10. It should be noted that Station 2 of the Transect cruise is station ALOHA. Although not shown in the figures, total LPS detected in the samples from the primary productivity, ATP, and deep casts of HOT 73 that were stored in the 1.5 ml polypropylene tubes were consistently higher than the total LPS detected in corresponding samples that were stored in 15 ml polypropylene tubes. When available, total LPS samples that were stored in the smaller tubes were preferentially used to calculate the amount of particulate LPS. Dissolved LPS accounted for approximately 50-60% of total LPS for the majority of the samples, but sometimes reached levels of 90% or more. The bulk of LPS detected was in the upper 200 meters of all profiles, and many of the sites had a peak of LPS at or around 100 meters.

The results of the biomass estimations based on the methods of flow cytometry, ATP, and LPS for the ATP cast of HOT 73 and station 2 of the Transect cruise are shown in Figures 11 and 12. On comparison of the two conversion factors used for the estimation based on the method of flow cytometry in relation to the other methods, 6.24 fg per cell (Christian and Karl, 1994) appears to give a more accurate estimation than 10 fg per cell.

The log of the quantities of particulate LPS were plotted against the log of the corresponding number of heterotrophic bacteria cells for the ATP cast of HOT 73 (Figure 13), Station 2 of the Transect cruise and published data collected by Watson *et al.* (1977) (Figure 14). The upper 200 m and the range of 200-1000 m were plotted separately for the two Station ALOHA casts,

revealing two distinct regression lines. Separation of these two depth ranges is justified in part by the large change in the amount of LPS detected above and below 200 m. Furthermore, the presence of an abundance of photoautotrophic organisms in the upper 200 m causes this portion of the water column to be quite distinct from the portion between 200 and 1000 m. For the HOT 73 ATP cast, the regression line through points from upper 200 m had a slope of 3.015 and a y-intercept of -15.138; the range from 200-1000 m returned a slope of 2.588 and a y-intercept of -11.613. The regression line through points from the upper 200 m of Station 2 on the Transect cruise had a slope of 3.995 and a y-intercept of -21.170; the range from 200-1000 m had a slope of 1.008 and a y-intercept of -4.266. Watson's data was all plotted together since depth ranges were not known; the regression line had a slope of 0.962 and a y-intercept of -2.480.

Discussion.

Methods of separation.

Based on the results of the separation experiment it appears that the method of centrifugation is more effective than filtration for removing particulate LPS from the sample water. the greater number of steps involved in the filtration procedure may have led to the introduction of contamination into the samples. Any contamination could have a large effect as the volume analyzed was so minute (20-40 µl). Centrifuging for longer periods of time had no apparent effect on the amount of dissolved LPS detected, so 10 minutes seems to be an adequate amount of time. However, the results of this experiment can only be assessed in relation to each other and there is no way to tell if the measured values of dissolved LPS reflect the actual amount in the sample water. Further experiments should involve taking flow

cytometry samples for cell enumeration from initial and processed samples in order to determine the efficiency of the removal of cells from the water.

Levels of dilution.

Although appropriate levels of dilution were determined at the beginning of this project, the spike returns varied with each different set of samples that were analyzed. This illustrates the variability that is inherent in the method, claimed to be approximately 25% (Associates of Cape Cod, Inc., Woods Hole, Mass.), and supports the need to monitor the accuracy of each sample set with spikes.

Effects of containers.

No conclusive trends of inhibition or enhancement of measured LPS associated with the type of storage container used could be determined from the container experiment. However, the amount of total LPS measured in the 15 ml Corning tubes was lower than the amount measured in the 1.5 ml microcentrifuge tubes at all times except for day 2, on which contamination may have been a factor. This occurrence was also noticed within the field profiles from which total LPS samples from both types of containers were analyzed. In these profiles, the amount of total LPS detected in the 15 ml tubes was often nearly equal to, and sometimes less than, the corresponding amount of dissolved LPS. If these values were accurate, it would mean that no particulate LPS was present at those points. However, analyses of flow cytometry samples from the HOT 73 ATP cast and the Transect Station 2 cast show the presence of heterotrophic bacteria at all depths, implying that the amounts of LPS measured in the 15 ml tubes may be in error. As noted earlier, Roslansky *et al.* (1991) reported that polypropylene containers could cause inhibition of detected LPS. Although the 15 ml and the 1.5 ml tubes used here were made by different manufacturers, they were both made of

polypropylene. If inhibition is occurring here, factors other than the type of plastic used, such as the manufacturing process or the size of the container, have caused it to be more pronounced in the 15 ml containers than the 1.5 ml microcentrifuge tubes. For future studies I recommend that total LPS samples be stored in the microcentrifuge tubes in order to avoid the possibility of inhibition of detectable LPS caused by the 15 ml tubes. This would also help to reduce the variability between the treatment of total samples and dissolved samples which are typically stored in microcentrifuge tubes.

Depth profiles.

All of the casts that were sampled from had similar particulate LPS profiles with a peak near 100 m and a considerable amount of variation in the upper 200 m. The profiles from the HOT 73 cruise showed peaks that were approximately twice as high as the peaks in the profiles from the Transect cruise. Although this may seem odd, it is supported by the ATP and flow cytometry data that have similar trends. The implied variation in bacterial biomass, however, is not supported by the ATP and flow cytometry measurements, suggesting that this variation may be due to the method. However, it is difficult to identify the aspect of the method that could cause such variation since profiles of both total and dissolved LPS track each other. It would be highly unlikely for errors in handling the samples to be duplicated for both the total and dissolved samples, and an error in the separation method would show a constant anomaly rather than a scattered one.

The methods of LPS and flow cytometry target the same population, yet the biomass estimations based on them differ by more than a factor of three. This large difference between biomass estimations could be a product of an inaccurate measurement of particulate LPS and/or an inaccurate conversion

factor for either method. If the measurement is in error it could be a product of the separation method, which has already been discussed. Watson *et al.* (1977) illustrated the correlation between particulate LPS concentrations and bacterial numbers with a scatter diagram similar to those shown in Figures 13 and 14.

A regression line with a slope of approximately one would support the idea of a constant relationship between LPS concentration and bacterial numbers. Only Watson's data points and those from 200-1000 m at Station 2 on the Transect cruise have regression lines with slopes near one. Although both of these regressions support the idea of a constant relationship between LPS concentration and bacterial numbers, the positions of the two lines on the plot are quite different. This implies that the conversion factor that was used by Watson *et al.* is not valid at station ALOHA.

The regression lines through both portions of the HOT 73 ATP cast and the upper 200 m portion of the Transect Station 2 cast (both station ALOHA) had steeper slopes with a relatively large increase in LPS concentration corresponding to a small increase in bacterial numbers. This implies that there may not be a constant relationship between LPS concentrations and bacterial numbers or that something else may be contributing to the LPS signal.

These plots raise many questions about the validity of the assumptions that the LPS method is based on, but more work needs to be done before any solid conclusions can be made. Although the slopes and positions of the regression lines through the shallow portions of both casts are very similar, the regression lines through the deeper portions of the two casts are different and don't support each other. It should also be noted that the regression lines through the deeper portions of the two casts were only based on 4-5 points on

the plot. Before the method of LPS is used further for estimation of marine bacterial biomass more work needs to be done to confirm the sources of LPS in marine waters and to establish a relevant C/LPS conversion factor.

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Figures.

1. Separation of Dissolved and Particulate LPS. Amounts of LPS, in ng/ml, detected after each method of separation.
2. Container effects over time. Figure on the top half of the page shows the amounts of LPS detected in samples stored in each type of container over time. Figure on the bottom half of the page shows the amounts of LPS detected in samples stored in the 15 ml polypropylene tube, the 1.5 ml microcentrifuge tube, and the dissolved sample stored in a 1.5 ml microcentrifuge tube.
3. Particulate LPS concentration vs. depth for the primary productivity cast of HOT 73.
4. Particulate LPS concentration vs. depth for the deep cast of HOT 73.
5. Particulate LPS concentration vs. depth for the ATP cast of HOT 73.
6. Particulate LPS concentration vs. depth for the cast at Station 1 of the Transect cruise.
7. Particulate LPS concentration vs. depth for the cast at Station 2 (a.k.a Station ALOHA) of the Transect cruise.
8. Particulate LPS concentration vs. depth for the cast at Station 3 of the Transect cruise.
9. Particulate LPS concentration vs. depth for the cast at Station 4 of the Transect cruise.
10. Particulate LPS concentration vs. depth for the cast at Station 5 of the Transect cruise.
11. Biomass estimations for the ATP cast on the HOT 73 cruise based on conversion factors of 250 for ATP, 6.35 for LPS, and 10 fg carbon per cell and 6.24 fg carbon per cell for flow cytometry.

12. Biomass estimations for the cast at Station 2 (a.ka. Station ALOHA) on the Transect cruise based on conversion factors of 250 for ATP, 6.35 for LPS, and 10 fg carbon per cell and 6.24 fg carbon per cell for flow cytometry.
13. Log of particulate LPS concentration vs. the log of bacterial numbers for the ATP cast of HOT 73. Regression lines were plotted through points from the upper 200 m (slope=3.015, y-int.=-15.138) and the points from 200-1000 m (slope=2.588, y-int.=-11.613).
14. Log of particulate LPS concentration vs. the log of bacterial numbers for the cast at Station 2 (a.k.a. Station ALOHA) on the Transect cruise and values published by Watson *et al.* (1977). Regression lines were plotted through points from the upper 200 m (slope=3.995, y-int.=-21.170), the points from 200-1000 m (slope=1.008, y-int.=-4.266), and the points published by Watson *et al.* (slope=0.9615, y-int.=-2.480).

Fig. 1

Separation of Dissolved and Particulate LPS
Filtration vs. Centrifugation

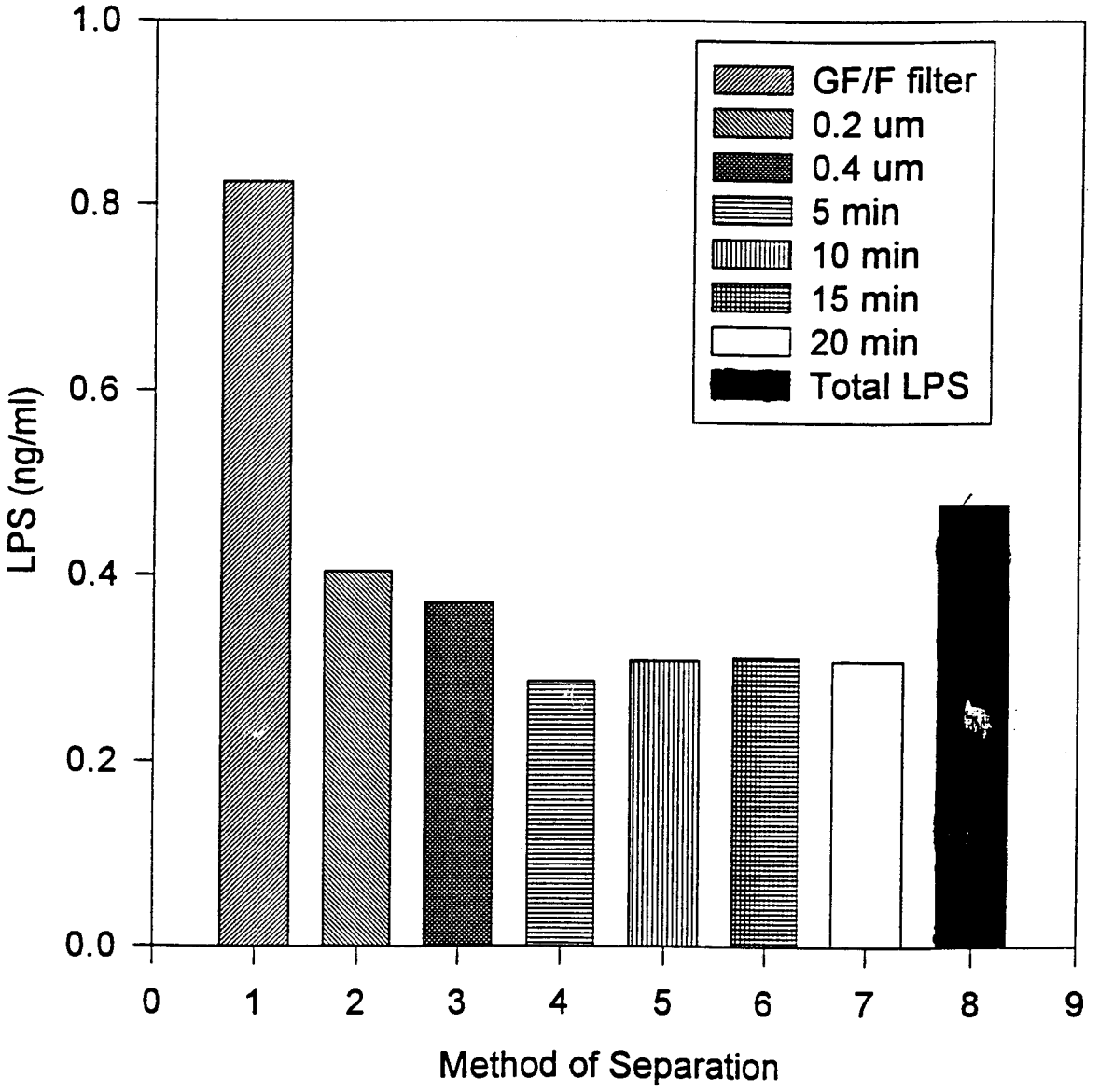
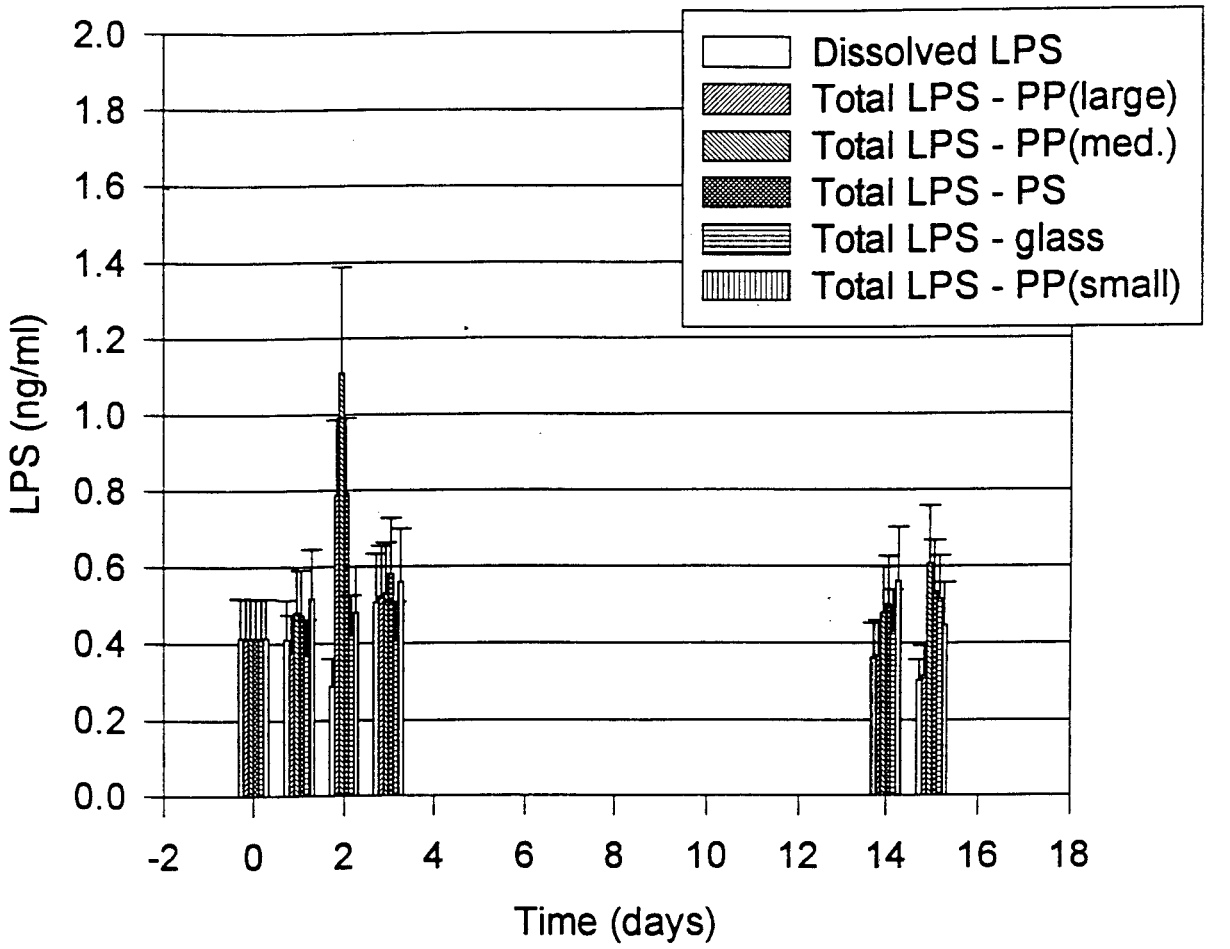


Fig. 2

Container effects over time



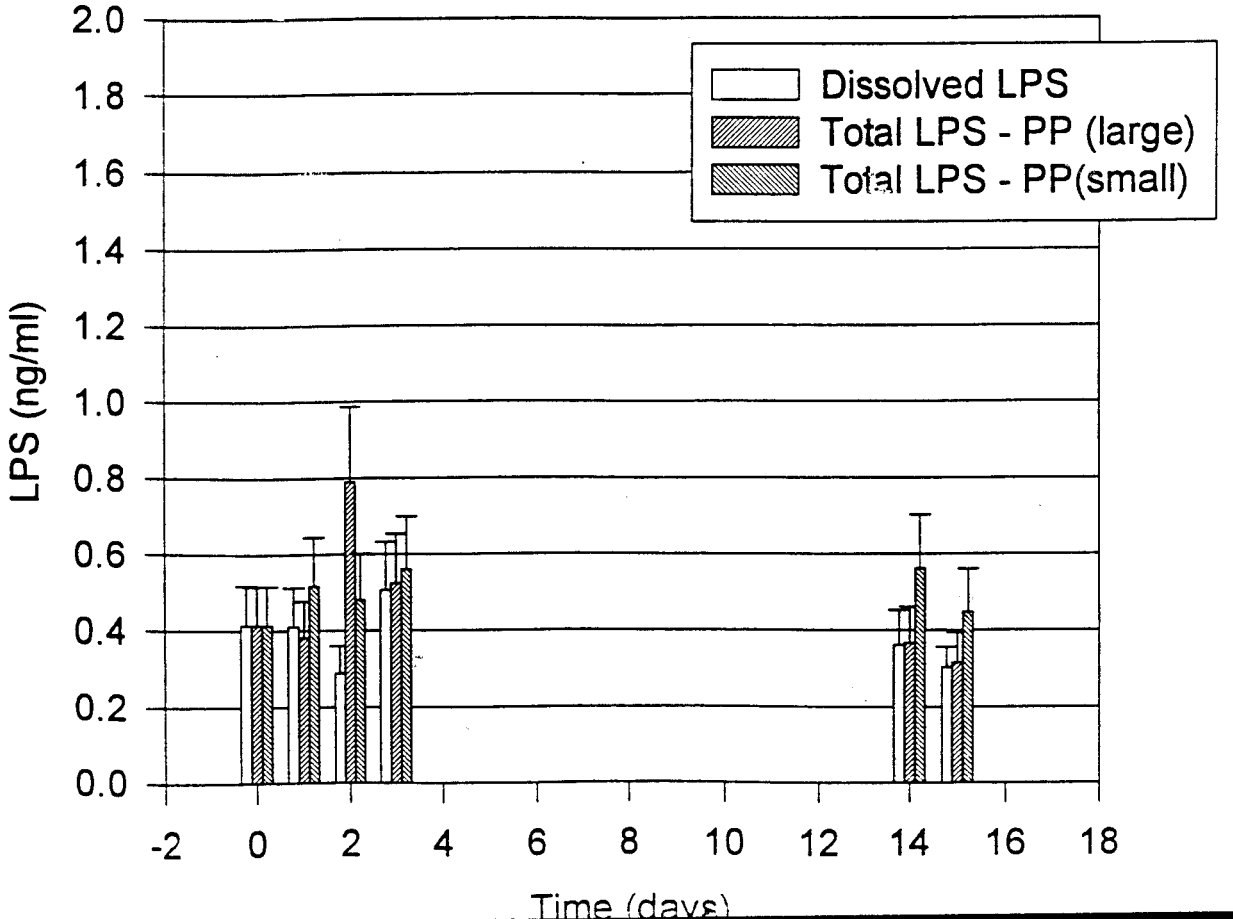


Fig. 3

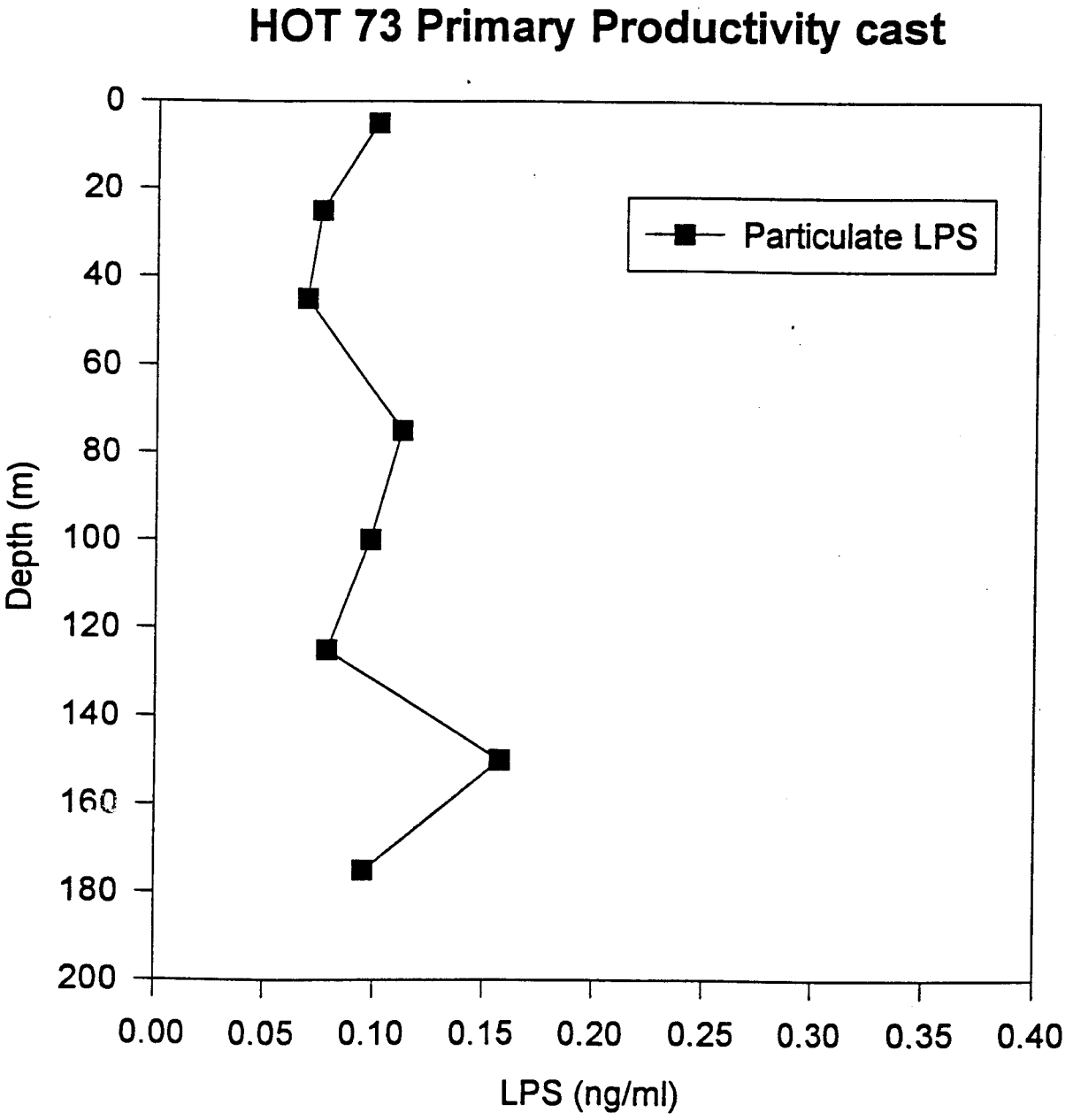
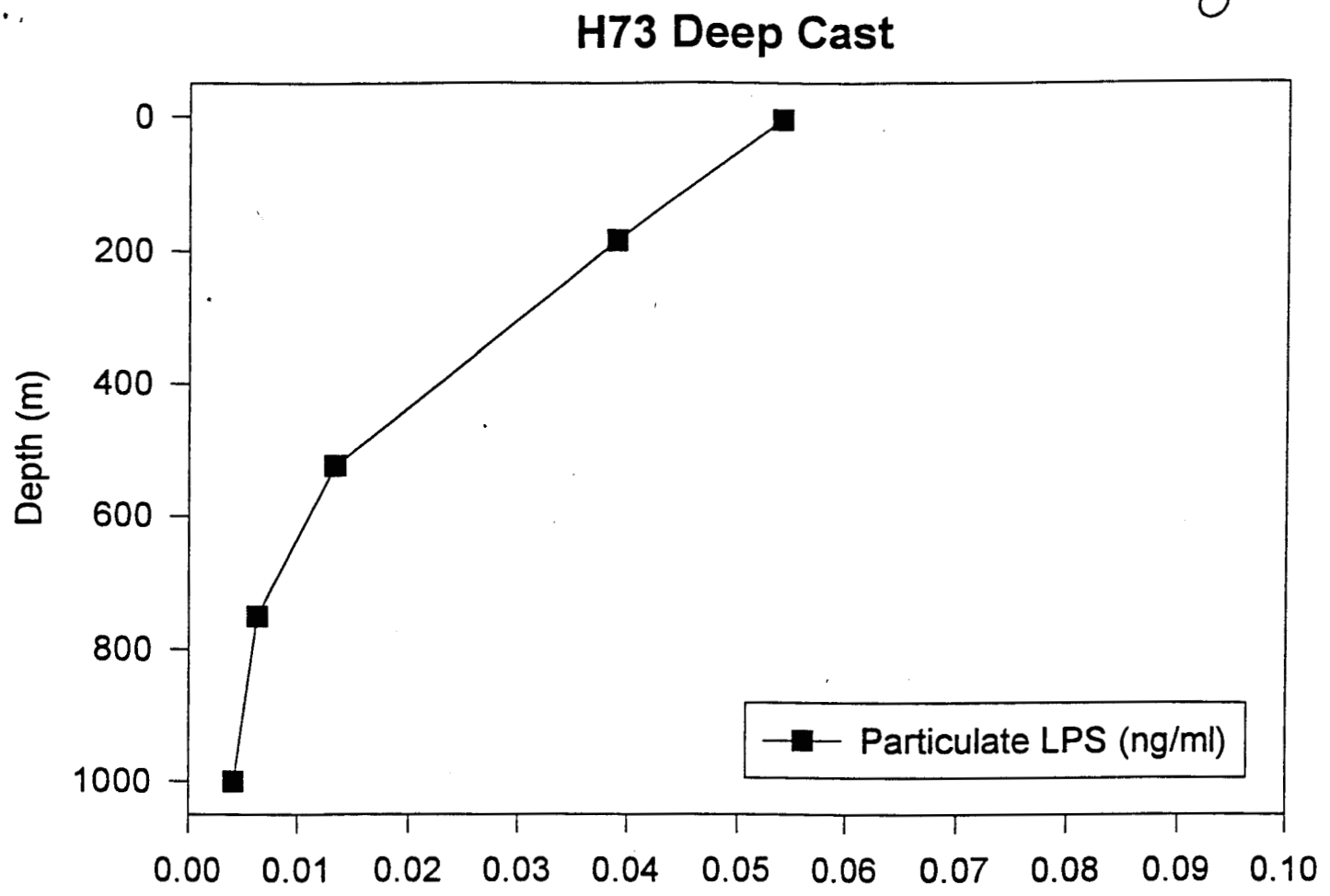

HOT 73 Primary Productivity cast
Particulate LPS
Depth (m)
0
20
40
60
80
100
120
140
160
180
200
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
LPS (ng/ml)

Fig. 4


H73 Deep Cast
Depth (m)
0
200
400
600
800
1000
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
Particulate LPS (ng/ml)

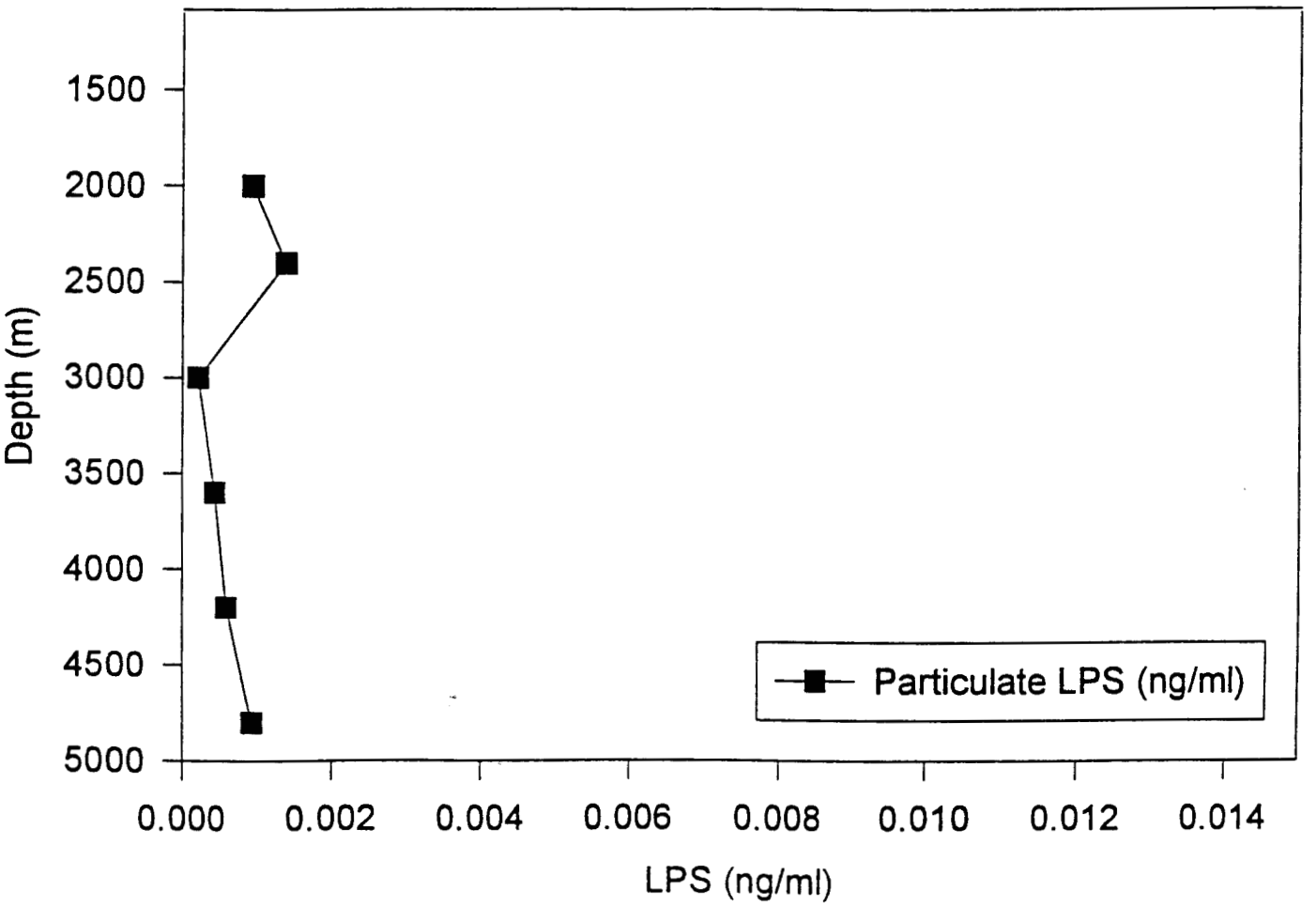

Depth (m)
1500
2000
2500
3000
3500
4000
4500
5000
0.000
0.002
0.004
0.006
0.008
0.010
0.012
0.014
LPS (ng/ml)
Particulate LPS (ng/ml)

Fig. 5

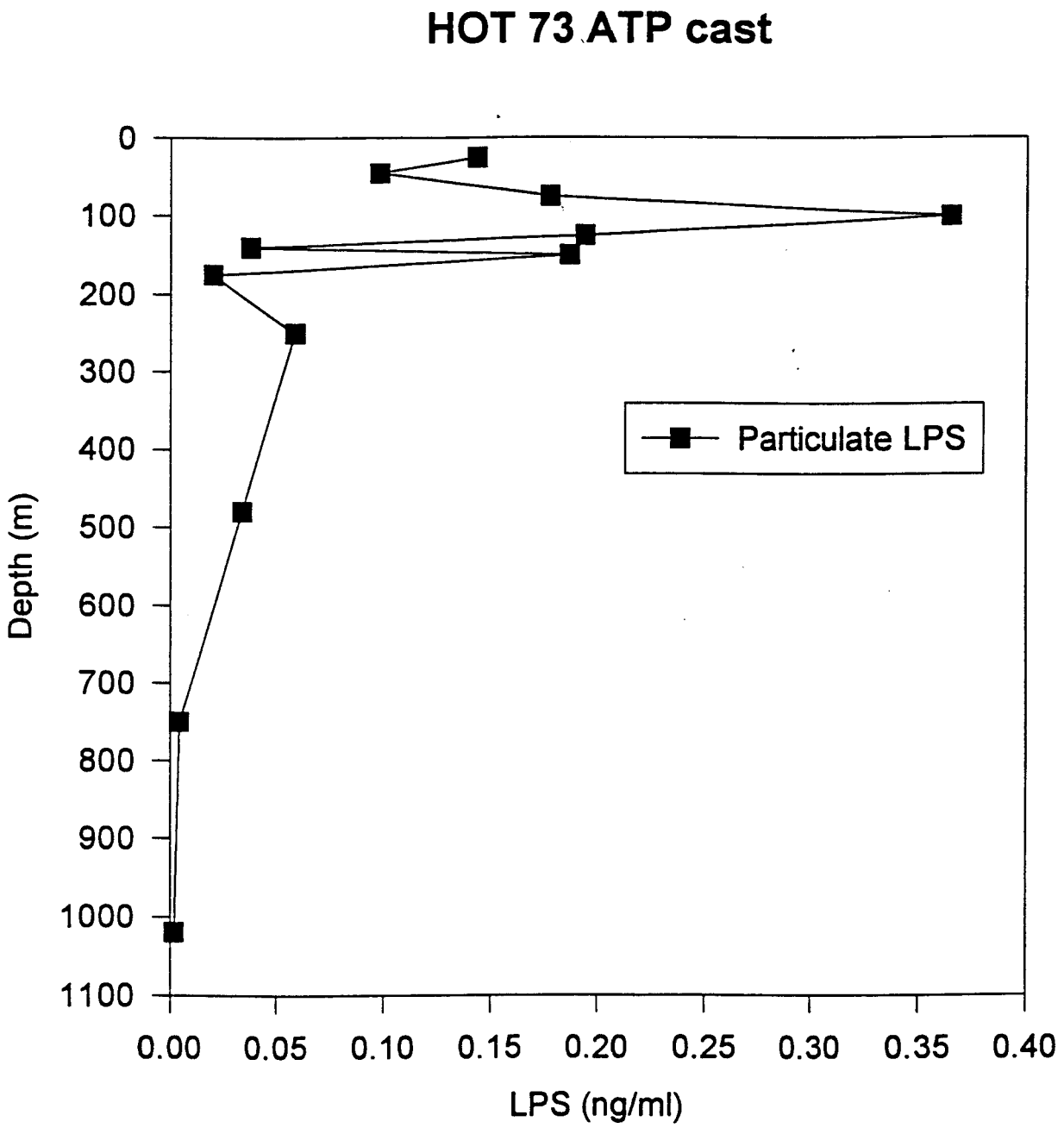

HOT 73 ATP cast
Depth (m)
0
100
200
300
400
500
600
700
800
900
1000
1100
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
LPS (ng/ml)
Particulate LPS

Fig. 6

ALOHA-CLIMAX Transect 1996
Station 1

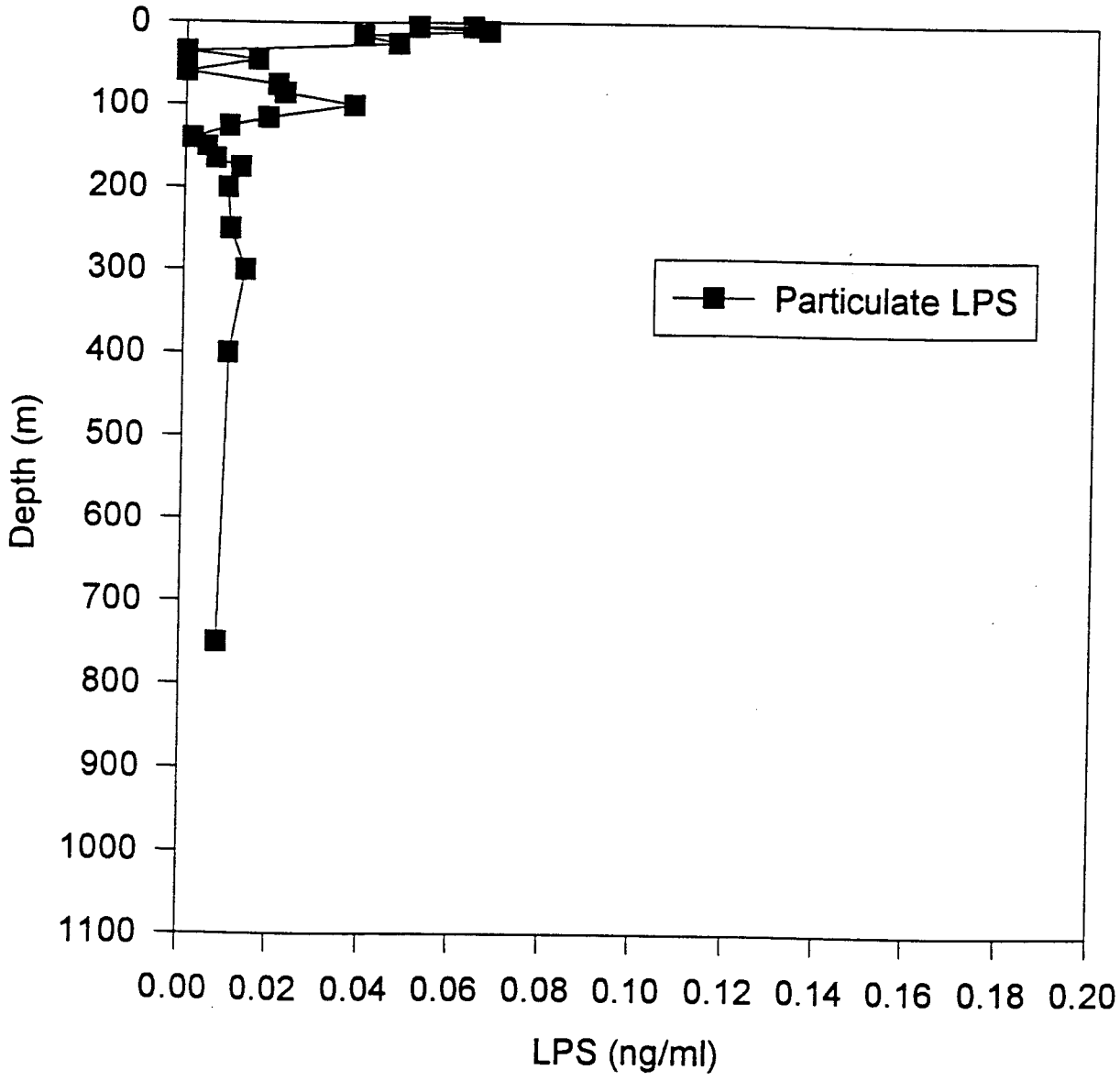


Fig. 7

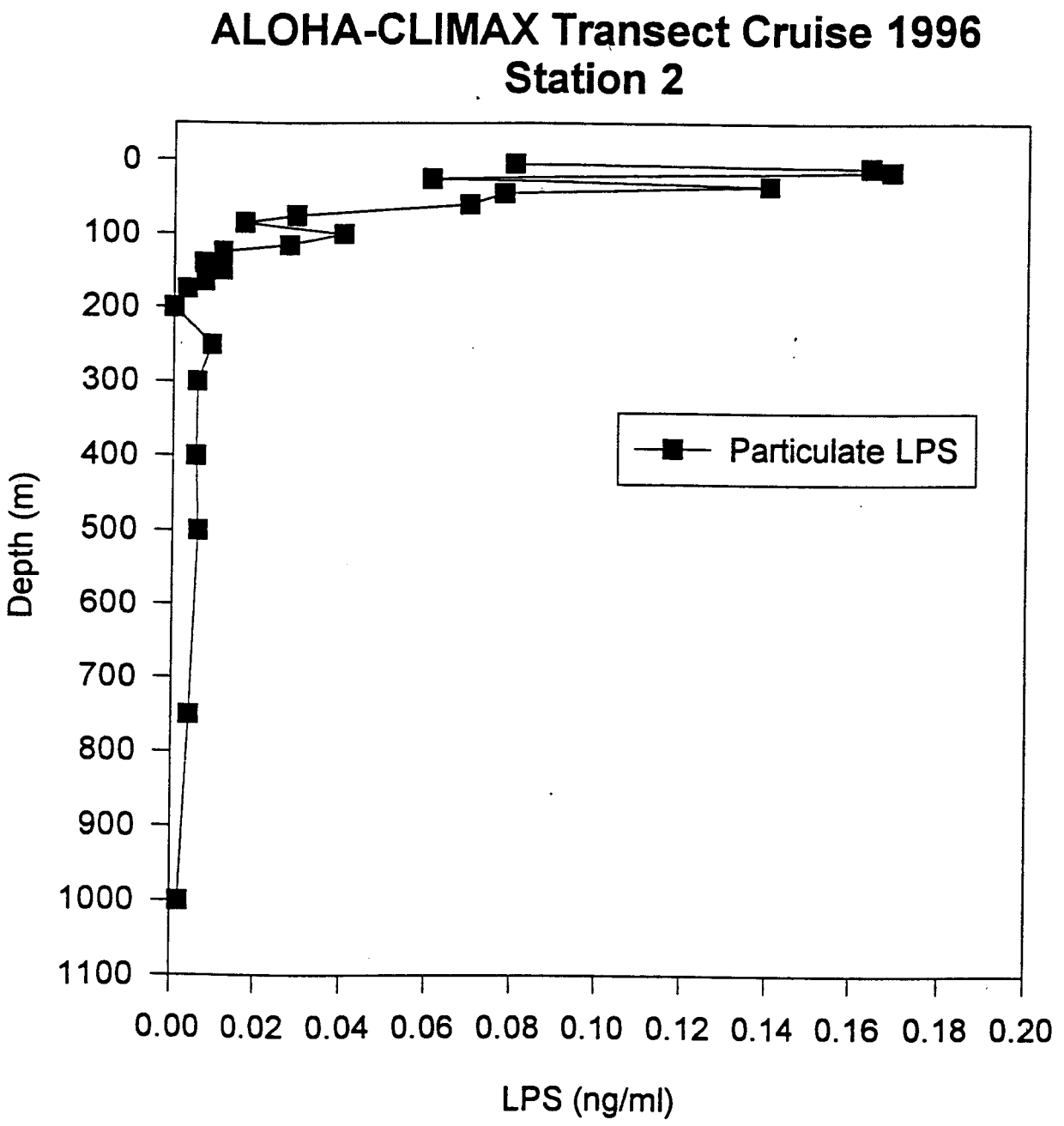

ALOHA-CLIMAX Transect Cruise 1996
Station 2
Depth (m)
0
100
200
300
400
500
600
700
800
900
1000
1100
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18
0.20
LPS (ng/ml)
Particulate LPS

Fig. 8

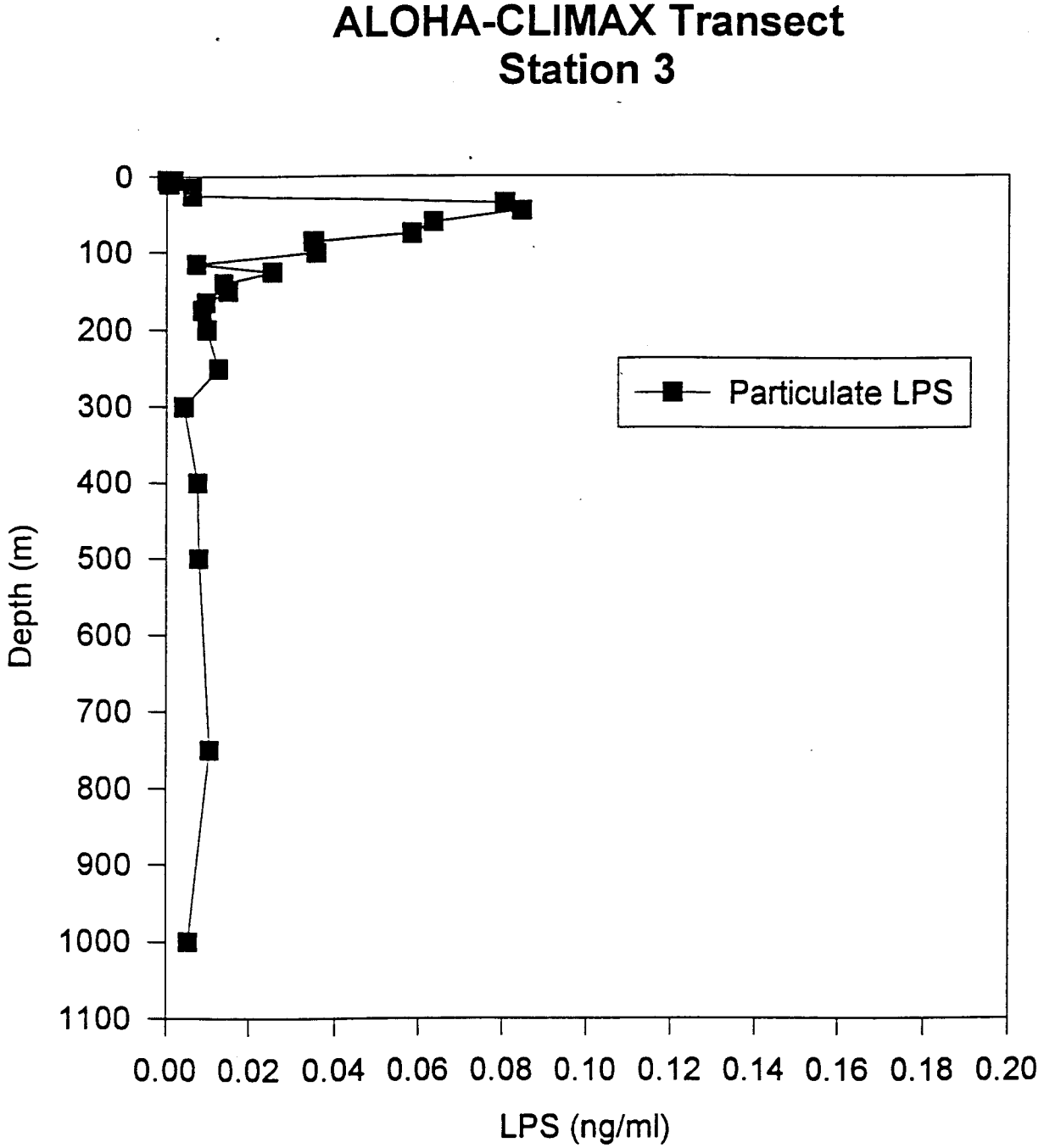

ALOHA-CLIMAX Transect
Station 3
Depth (m)
0
100
200
300
400
500
600
700
800
900
1000
1100
Particulate LPS
0.00 0.02 0.04 0.06 0.08 0.10 0.12 0.14 0.16 0.18 0.20
LPS (ng/ml)

Fig. 9

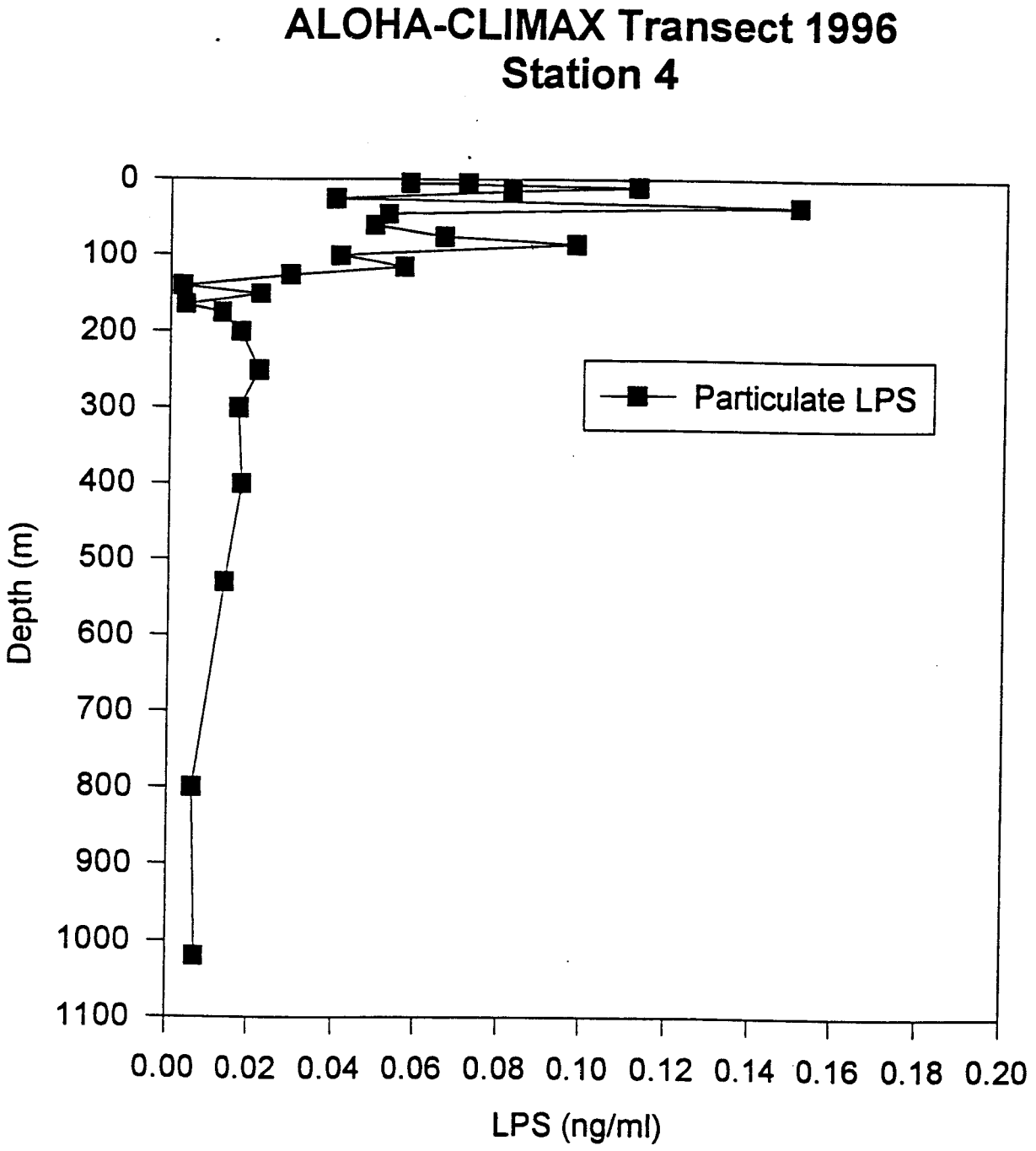

ALOHA-CLIMAX Transect 1996
Station 4
Depth (m)
0
100
200
300
400
500
600
700
800
900
1000
1100
Particulate LPS
0.00 0.02 0.04 0.06 0.08 0.10 0.12 0.14 0.16 0.18 0.20
LPS (ng/ml)

Fig. 10

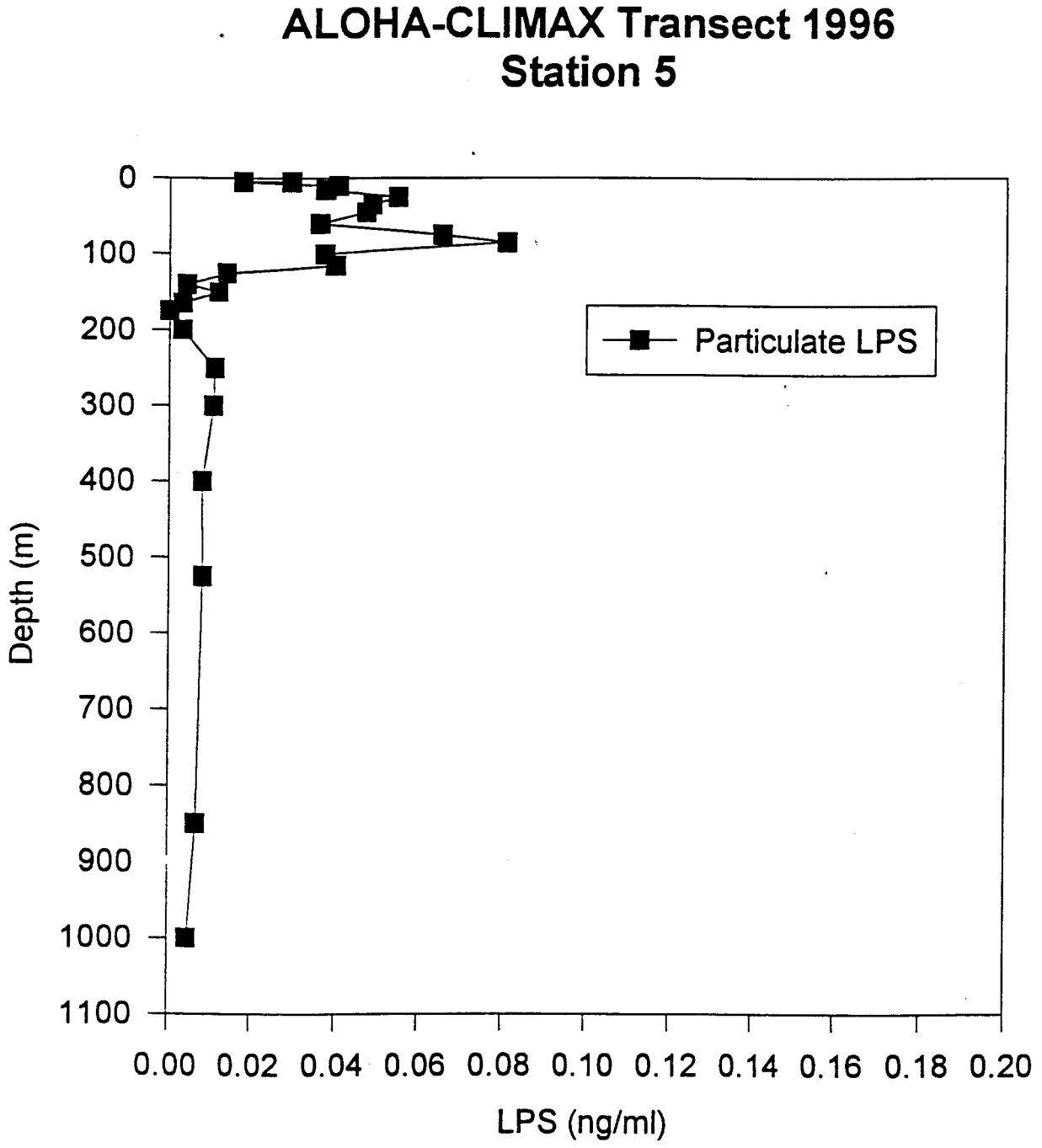
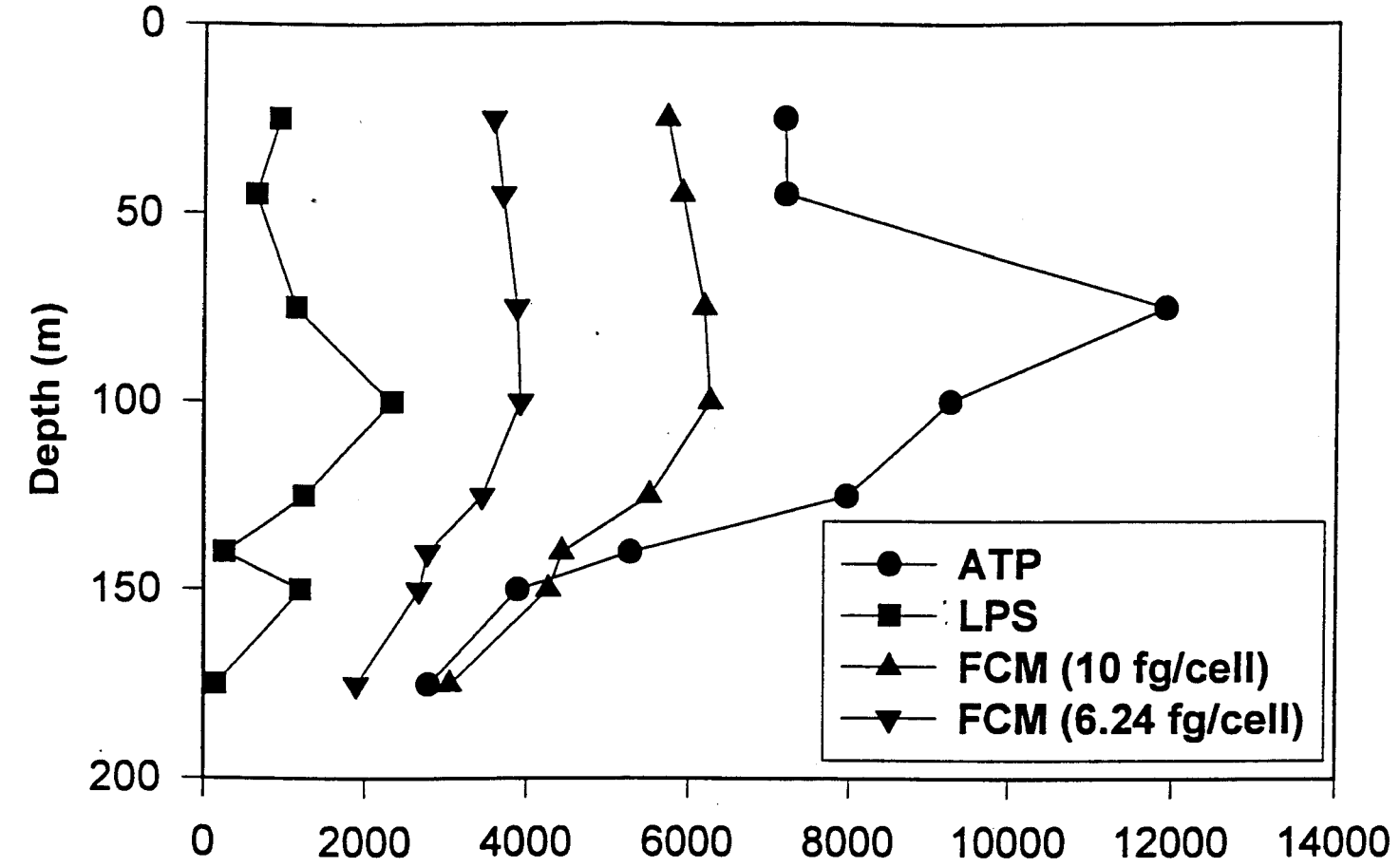

ALOHA-CLIMAX Transect 1996
Station 5
Depth (m)
0
100
200
300
400
500
600
700
800
900
1000
1100
Particulate LPS
0.00 0.02 0.04 0.06 0.08 0.10 0.12 0.14 0.16 0.18 0.20
LPS (ng/ml)

Fig. 11

Biomass Estimations
ATP cast, HOT-73



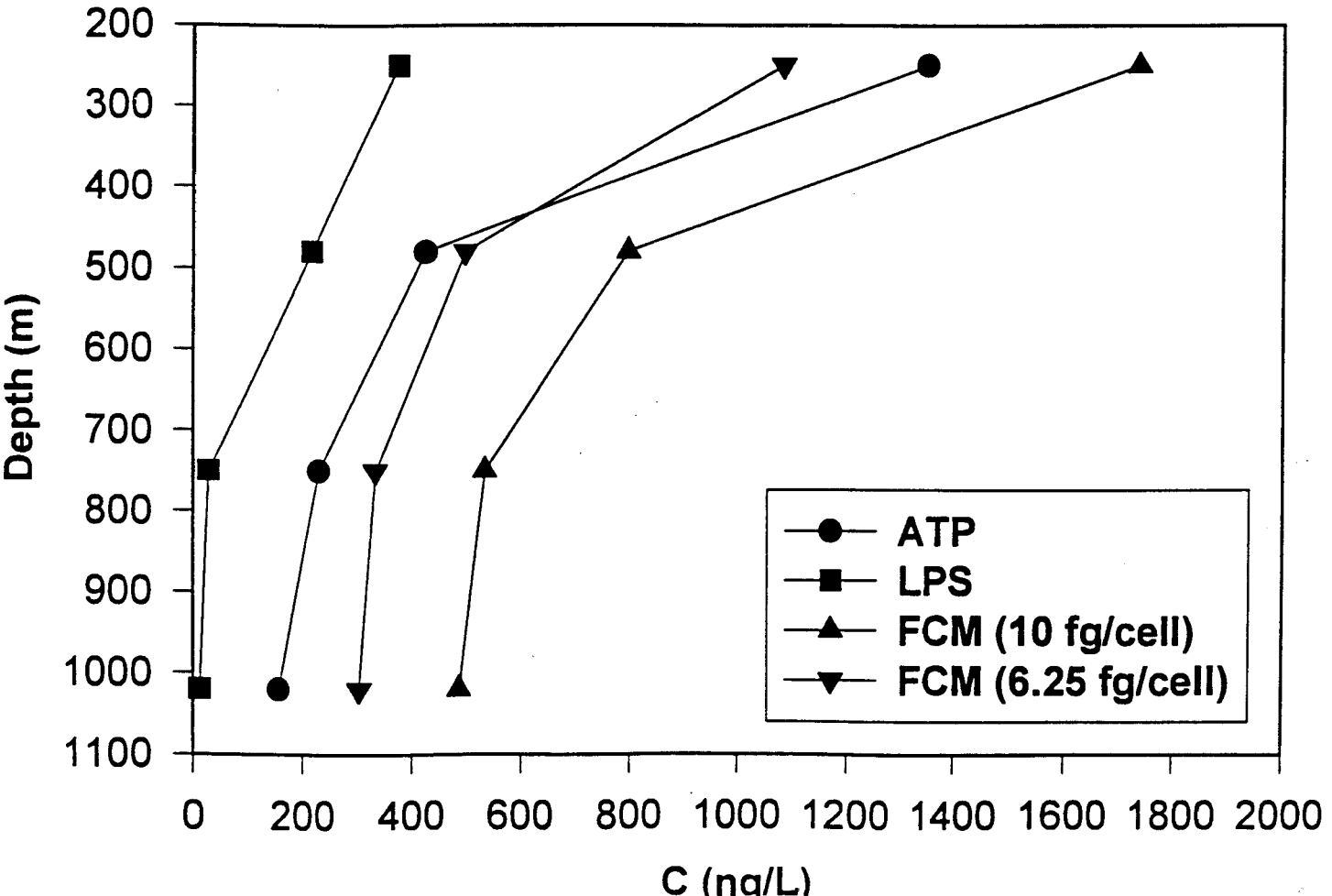


Fig. 12

Biomass Estimations
Station 2, Transect Cruise

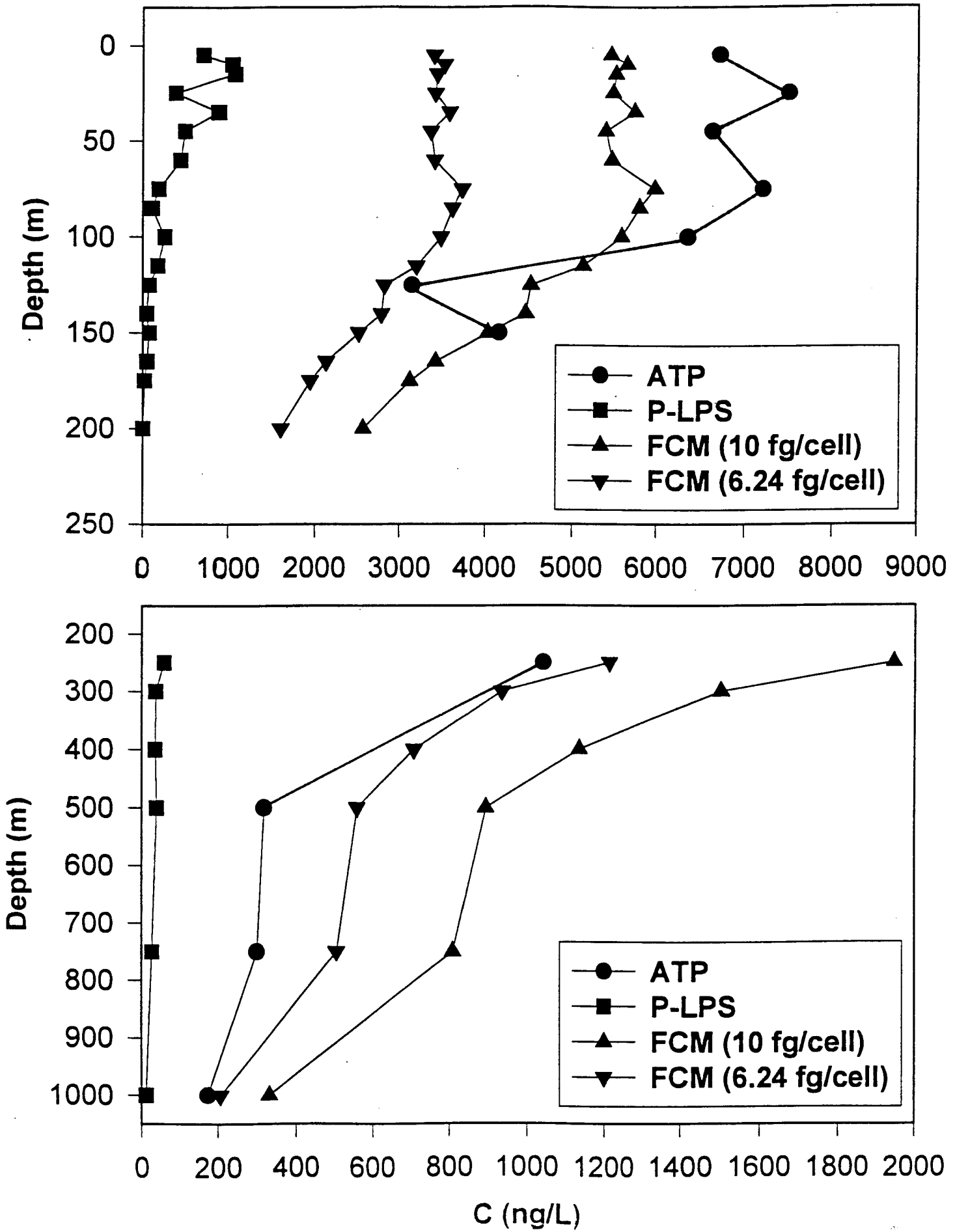


Fig. 13

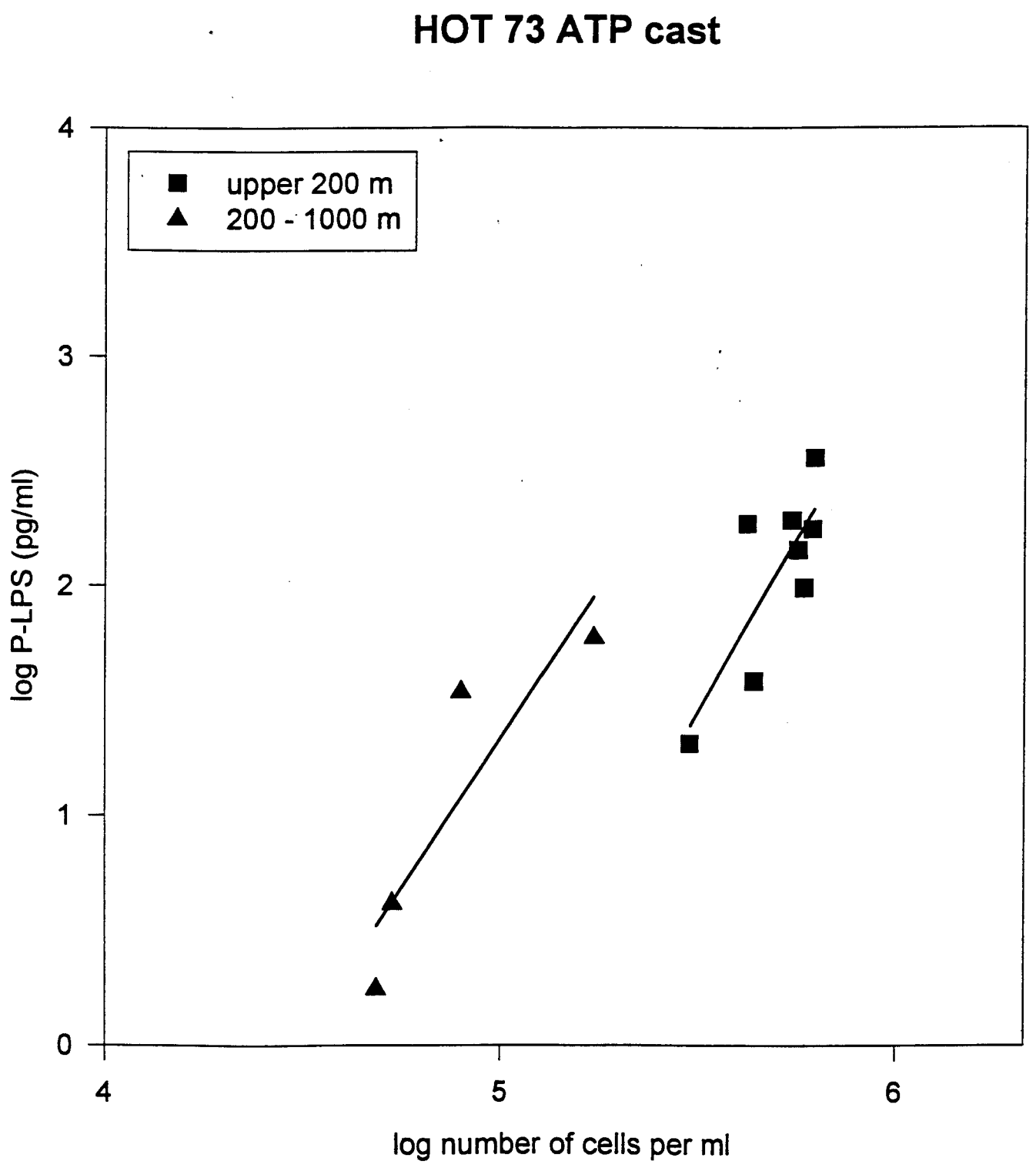
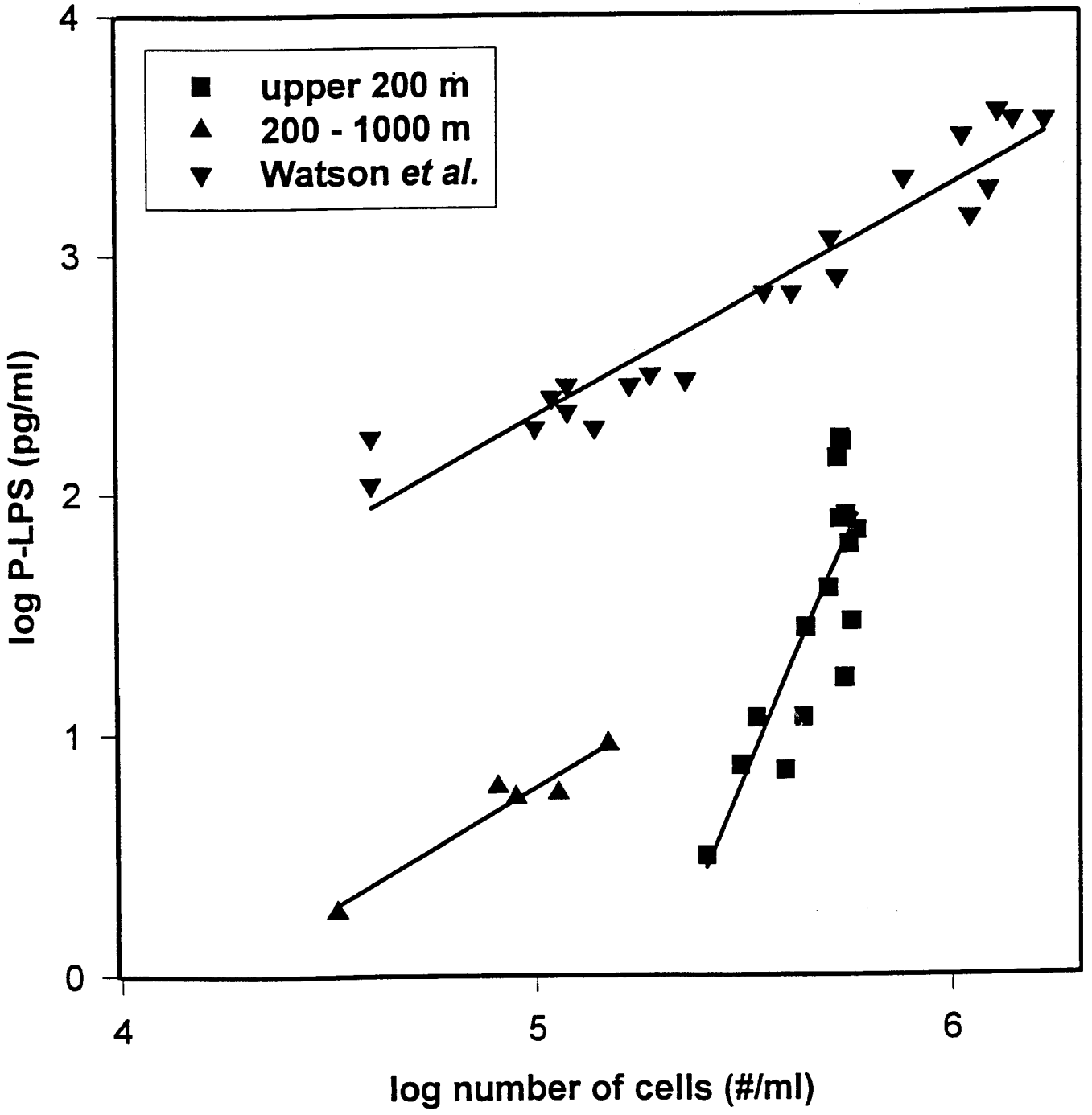

HOT 73 ATP cast
upper 200 m
200 - 1000 m
log P-LPS (pg/ml)
0
1
2
3
4
4
5
6
log number of cells per ml

Fig. 14

Cell Number vs. Quantity of LPS
Station 2, Transect Cruise



	-1-	-2-	-3-	-4-	-5-
	Depth1				P-LPS 1
1	Depth	Total (sma	Dissolved	% diss	Particulat
2	4800.0000	1.8150e-3	8.6300e-4	0.4755	9.5200e-4
3	4200.0000	1.2950e-3	6.9000e-4	0.5328	6.0500e-4
4	3600.0000	1.2490e-3	8.0850e-4	0.6473	4.4050e-4
5	3000.0000	7.5250e-4	5.3900e-4	0.7163	2.1350e-4
6	2400.0000	2.0600e-3	6.5850e-4	0.3197	1.4015e-3
7	2000.0000	2.0400e-3	1.0835e-3	0.5311	9.5650e-4
8	1600.0000	3.7950e-3			
9	1000.0000	6.1300e-3	1.9450e-3	0.3173	4.1850e-3
10	750.0000	0.0101	3.7550e-3	0.3718	6.3450e-3
11	525.0000	0.0183	5.0450e-3	0.2757	0.0133
12	185.0000	0.0622	0.0233	0.3741	0.0389
13	5.0000	0.1220	0.0681	0.5582	0.0539

HOT IS Primary Productivity cast

-1- -2- -3- -4- -5-

	Depth	Total (sma	Dissolved	% diss	Particulat
2	175.0000	0.1550	0.0598	0.3858	0.0952
3	150.0000	0.3210	0.1635	0.5093	0.1575
4	125.0000	0.1185	0.0402	0.3392	0.0783
5	100.0000	0.1550	0.0571	0.3684	0.0979
6	75.0000	0.2595	0.1475	0.5684	0.1120
7	45.0000	0.1650	0.0965	0.5845	0.0685
8	25.0000	0.2220	0.1470	0.6622	0.0750
9	5.0000	0.2120	0.1120	0.5283	0.1000

A:\ATP.SPW HOT 73 ATP cast

	-1- Depth	-2- T-LPS(sm)	-3- diss	-4- % diss	-5- -LPS(ng/ml	-6- lpsC (ng/L)
1	1020.0000	5.2800e-3	3.5250e-3	0.6676	1.7550e-3	11.1443
2	750.0000	7.4700e-3	3.3300e-3	0.4458	4.1400e-3	26.2890
3	480.0000	0.0397	5.4750e-3	0.1379	0.0342	217.3288
4	250.0000	0.0713	0.0123	0.1725	0.0590	374.6500
5	175.0000	0.0410	0.0206	0.5031	0.0204	129.5400
6	150.0000	0.2075	0.0204	0.0983	0.1871	1188.0850
7	140.0000	0.0627	0.0245	0.3911	0.0382	242.5700
8	125.0000	0.2144	0.0200	0.0931	0.1944	1234.4400
9	100.0000	0.4030	0.0383	0.0950	0.3647	2315.8450
10	75.0000	0.2395	0.0616	0.2572	0.1779	1129.6650
11	45.0000	0.1545	0.0564	0.3650	0.0981	622.9350
12	25.0000	0.1985	0.0547	0.2753	0.1438	913.1300

	TP-C (ng/L	hbact(#/ml)
1	155.2500	4.8725e+4
2	231.0000	5.3486e+4
3	424.5000	7.9747e+4
4	1351.7500	1.7377e+5
5	2776.5000	3.0373e+5
6	3890.0000	4.2608e+5
7	5278.7500	4.4309e+5
8	7961.7500	5.5114e+5
9	9252.7500	6.2676e+5
10	1.1901e+4	6.1913e+5
11	7204.2500	5.9083e+5
12	7186.7500	5.7160e+5

A:\ACI_1.DAT

Transect Cruise Station 1

	-1- Depth	-2- Total-a	-3- diss-a	-4- % diss	-5- part-a
1	750.0000	0.0102	1.9700e-3	0.1935	8.2300e-3
2	400.0000	0.0254	0.0152	0.5974	0.0102
3	300.0000	0.0206	6.7500e-3	0.3277	0.0139
4	250.0000	0.0193	8.9700e-3	0.4648	0.0103
5	200.0000	0.0189	9.1400e-3	0.4831	9.7600e-3
6	175.0000	0.0280	0.0155	0.5546	0.0125
7	165.0000	0.0283	0.0215	0.7597	6.8000e-3
8	150.0000	0.0247	0.0199	0.8073	4.8000e-3
9	140.0000	0.0235	0.0220	0.9360	1.5000e-3
10	125.0000	0.0314	0.0216	0.6874	9.8000e-3
11	115.0000	0.0448	0.0263	0.5877	0.0185
12	100.0000	0.0645	0.0273	0.4233	0.0372
13	85.0000	0.0899	0.0678	0.7540	0.0221
14	75.0000	0.1010	0.0804	0.7960	0.0206
15	60.0000	0.0649	0.0754	1.1618	0.0000
16	45.0000	0.0796	0.0636	0.7990	0.0160
17	35.0000	0.0613	0.0929	1.5155	0.0000
18	25.0000	0.1197	0.0728	0.6084	0.0469
19	15.0000	0.1235	0.0844	0.6834	0.0391
20	10.0000	0.1410	0.0741	0.5255	0.0669
21	5.0000	0.1107	0.0594	0.5368	0.0513
22	5.0000	0.1264	0.0631	0.4994	0.0633

Transect Cruise Station 2

	-1- Depth	-2- Total-lg	-3- Dissolved	-4- % diss	-5- Particulate
1	1000.0000	5.5450e-3	3.7100e-3	0.6691	1.8350e-3
2	750.0000	0.0101	6.0300e-3	0.5953	4.1000e-3
3	500.0000	0.0154	9.3400e-3	0.6085	6.0100e-3
4	400.0000	0.0115	6.0650e-3	0.5274	5.4350e-3
5	300.0000	0.0171	0.0115	0.6696	5.6500e-3
6	250.0000	0.0247	0.0156	0.6329	9.0500e-3
7	200.0000	0.0151	0.0170	1.1262	0.0000
8	175.0000	0.0228	0.0197	0.8637	3.1000e-3
9	165.0000	0.0282	0.0208	0.7389	7.3500e-3
10	150.0000	0.0299	0.0183	0.6131	0.0116
11	140.0000	0.0304	0.0234	0.7697	7.0000e-3
12	125.0000	0.0400	0.0284	0.7100	0.0116
13	115.0000	0.0469	0.0196	0.4168	0.0274
14	100.0000	0.0638	0.0240	0.3759	0.0398
15	85.0000	0.0602	0.0434	0.7201	0.0169
16	75.0000	0.0682	0.0392	0.5748	0.0290
17	60.0000	0.1033	0.0338	0.3269	0.0695
18	45.0000	0.1065	0.0290	0.2718	0.0776
19	35.0000	0.1670	0.0272	0.1626	0.1398
20	25.0000	0.0900	0.0294	0.3268	0.0606
21	15.0000	0.2065	0.0382	0.1850	0.1683
22	10.0000	0.1988	0.0351	0.1764	0.1637
23	5.0000	0.1263	0.0463	0.3661	0.0801

1	Depth	Total	Dissolved	% diss	Particulat
2	1000.0000	7.3500e-3	2.1400e-3	0.2912	5.2100e-3
3	750.0000	0.0132	2.7600e-3	0.2091	0.0104
4	500.0000	0.0139	6.1200e-3	0.4403	7.7800e-3
5	400.0000	0.0127	5.2350e-3	0.4122	7.4650e-3
6	300.0000	9.8000e-3	5.7100e-3	0.5827	4.0900e-3
7	250:0000	0.0186	6.0550e-3	0.3264	0.0125
8	200.0000	0.0157	5.9850e-3	0.3824	9.7150e-3
9	175.0000	0.0163	7.7600e-3	0.4761	8.5400e-3
10	165.0000	0.0186	9.1950e-3	0.4930	9.4410e-3
11	150.0000	0.0249	0.0100	0.4002	0.0149
12	140.0000	0.0309	0.0171	0.5534	0.0138
13	125.0000	0.0405	0.0151	0.3724	0.0254
14	115.0000	0.0388	0.0317	0.8170	7.1000e-3
15	100.0000	0.0515	0.0160	0.3107	0.0355
16	85.0000	0.0725	0.0376	0.5197	0.0349
17	75.0000	0.1026	0.0443	0.4320	0.0583
18	60.0000	0.1235	0.0600	0.4854	0.0635
19	45.0000	0.2845	0.2000	0.7030	0.0845
20	35.0000	0.3805	0.3000	0.7884	0.0805
21	25.0000	0.2530	0.2470	0.9763	6.0000e-3
22	15.0000	0.2075	0.2015	0.9711	6.0000e-3
23	10.0000	0.2915	0.2910	0.9983	5.0000e-4
24	5.0000	0.2745	0.2730	0.9945	1.5000e-3
25	5.0000	0.2090	0.2850	1.3636	0.0000

1	Depth	Total	Dissolved	% diss	Particulat
2	1020.0000	0.0113	4.4200e-3	0.3929	6.8800e-3
3	800.0000	0.0122	6.2500e-3	0.5144	5.9500e-3
4	530.0000	0.0246	0.0109	0.4411	0.0137
5	400.0000	0.0254	7.6250e-3	0.3008	0.0178
6	300.0000	0.0269	0.0100	0.3736	0.0169
7	250.0000	0.0327	0.0109	0.3352	0.0218
8	200.0000	0.0342	0.0169	0.4942	0.0173
9	175.0000	0.0384	0.0258	0.6706	0.0126
10	165.0000	0.0266	0.0230	0.8663	3.6000e-3
11	150.0000	0.0568	0.0349	0.6141	0.0219
12	140.0000	0.0516	0.0488	0.9448	2.8000e-3
13	125.0000	0.0688	0.0398	0.5785	0.0290
14	115.0000	0.0937	0.0374	0.3994	0.0563
15	100.0000	0.0954	0.0547	0.5729	0.0407
16	85.0000	0.1540	0.0562	0.3649	0.0978
17	75.0000	0.1225	0.0565	0.4612	0.0660
18	60.0000	0.1144	0.0654	0.5712	0.0490
19	45.0000	0.1150	0.0627	0.5448	0.0523
20	35.0000	0.2470	0.0958	0.3879	0.1512
21	25.0000	0.2190	0.1795	0.8196	0.0395
22	15.0000	0.2540	0.1720	0.6772	0.0820
23	10.0000	0.2845	0.1720	0.6046	0.1125
24	5.0000	0.2665	0.2090	0.7842	0.0575
25	5.0000	0.2565	0.1850	0.7212	0.0715

Transect Cruise
Station 5

-1- -2- -3- -4- -5-

	Depth	Total	Dissolved	% diss	Particulat
2	1000.0000	7.7750e-3	3.1800e-3	0.4090	4.5950e-3
3	850.0000	0.0111	4.4800e-3	0.4038	6.6200e-3
4	525.0000	0.0167	8.5700e-3	0.5147	8.1300e-3
5	400.0000	0.0170	9.0350e-3	0.5315	7.9650e-3
6	300.0000	0.0165	5.7700e-3	0.3508	0.0107
7	250.0000	0.0266	0.0155	0.5838	0.0111
8	200.0000	0.0171	0.0140	0.8158	3.1000e-3
9	175.0000	0.0181	0.0192	1.0635	0.0000
10	165.0000	0.0203	0.0172	0.8469	3.1000e-3
11	150.0000	0.0297	0.0178	0.6003	0.0119
12	140.0000	0.0244	0.0202	0.8275	4.2000e-3
13	125.0000	0.0313	0.0174	0.5575	0.0139
14	115.0000	0.0698	0.0301	0.4305	0.0397
15	100.0000	0.0685	0.0313	0.4569	0.0372
16	85.0000	0.1280	0.0472	0.3684	0.0808
17	75.0000	0.1170	0.0515	0.4402	0.0655
18	60.0000	0.1485	0.1125	0.7576	0.0360
19	45.0000	0.1550	0.1080	0.6968	0.0470
20	35.0000	0.0939	0.0455	0.4848	0.0484
21	25.0000	0.0986	0.0439	0.4455	0.0547
22	15.0000	0.0834	0.0461	0.5522	0.0373
23	10.0000	0.0752	0.0349	0.4641	0.0403
24	5.0000	0.0718	0.0540	0.7514	0.0178
25	5.0000	0.0800	0.0506	0.6325	0.0294

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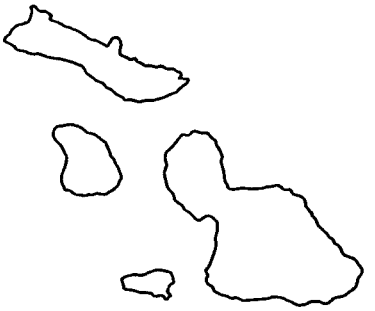

Research Experiences for Undergraduates

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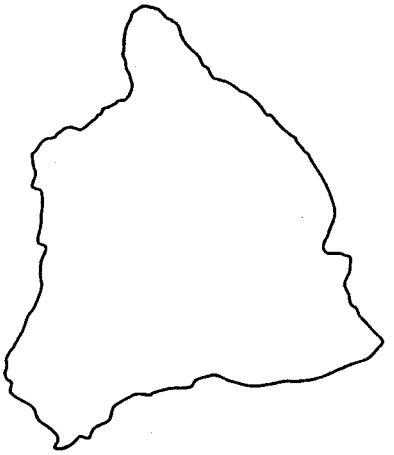

School of Ocean & Earth Science & Technology
University of Hawai'i at Mānoa

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